Altered Laminin 5 Expression Due to Mutations in the Gene Encoding the β3 Chain (LAMB3) in Generalized Atrophic Benign Epidermolysis Bullosa

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The anchoring filament component laminin 5 (kalinin/nicein) is a candidate protein for mutations in some hereditary blistering skin disorders. In this study, laminin 5 expression was assessed in a family with generalized atrophic benign epidermolysis bullosa, a non-lethal variant of the junctional form of epidermolysis bullosa. Immunofluorescence microscopy of the skin basement-membrane zone with a monoclonal antibody (GB3) revealed reduced anti-laminin 5 staining compared to normal controls. The labeling, when examined by immunoelectron microscopy, was present within the lower lamina lucida, immediately below the plane of blister formation. Numerous hemidesmosomes and well-formed anchoring filaments were seen on transmission electron microscopy. Polymerase chain reaction amplification of genomic DNA encoding the β3 subunit (LAMB3) of laminin 5, heteroduplex analysis of the polymerase chain reaction products, and nucleotide sequencing of the heteroduplexes revealed two putative mutations within the LAMB3 gene; these consisted of a premature termination codon in exon 3 and a missense mutation in exon 7. Exons 3 and 7 encode part of domain VI of the laminin 5 β3 chain short arm. This globular domain of the protein has been postulated to have an important function in the interaction of laminin 5 with other structural components of the basement membrane zone, such as laminin 6 (K-laminin). Thus the mutations delineated in this family may have a critical pathogenetic significance in reducing adhesion between the epidermis and the dermis. ***Key words: junctional epidermolysis bullosa/kalinin/nicein. J Invest Dermatol 104:467–474, 1995***

The mechanobullous disorder, junctional epidermolysis bullosa (JEB), is a heterogeneous condition with descriptions of both lethal and non-lethal variants [1–3]. Generalized atrophic benign epidermolysis bullosa (GABEB) is a rare form of non-lethal JEB and was first reported using this nomenclature by Hintner and Wolff [4]. Apart from skin fragility, it is associated with widespread atrophic skin changes, alopecia, reduced axillary and pubic hair, dysplastic teeth, and dystrophic nails. Several additional cases of GABEB with similar clinical features, and with ultrastructural evidence of blistering within the lamina lucida and variable changes in hemidesmosome and anchoring filament morphology, have been reported [5–9].

Recently, evidence has emerged invoking the three genes that encode the anchoring filament protein laminin 5 as candidate genes for JEB. In particular, immunofluorescence studies using antibodies to laminin 5 have shown reduced or absent skin basement membrane zone labeling in JEB [10–12]. Laminin 5 consists of three subunit polypeptide chains, α3, β3, and γ2, encoded by distinct genes LAMA3, LAMB3, and LAMC2, respectively [13]. These polypeptide chains (formerly known as nicein/kalinin A, B1, and B2 chains) form a heterotrimeric molecule, and all three are required in the assembly of functional laminin 5 macromolecules. Specific mutations in both the LAMB3 and LAMC2 genes have been reported in patients with either lethal or non-lethal JEB phenotypes [14–16].

In this study, we have searched for mutations in laminin 5 in a family containing three affected members with GABEB. Skin samples were assessed by electron microscopy and by immunofluorescence and pre-embedding immunogold electron microscopy, using the GB3 monoclonal antibody (anti-laminin 5) [10]. Polymerase chain reaction (PCR) amplification of genomic DNA segments and heteroduplex analysis was used to screen for mutations, followed by nucleotide sequencing of any observed heteroduplex. Sequence abnormalities were then confirmed by restriction endonuclease digestion or allele-specific oligonucleotide (ASO) hybridization. Transcript levels for LAMB3 were also assessed from cultured keratinocytes derived from two affected patients. Reverse transcription (RT) PCR followed by hybridization with oligonucleotide probes to the relevant exon borders was performed to assess cDNA structure.

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Abbreviations: ASO, allele-specific oligonucleotide hybridization; GABEB, generalized atrophic benign epidermolysis bullosa; PTC, premature termination codon.

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MATERIALS AND METHODS

Patient Data Complete clinical examination of the affected members of the family was carried out by at least two of the authors. Two 6-mm punch biopsies of skin were taken under local anesthesia from the upper arm in all the affected family members. Non-lesional skin that had been rubbed gently with an eraser for 5–10 seconds was sampled. One biopsy specimen was divided for immunofluorescence, electron microscopy, and immunoelectron microscopy studies. The other was used to initiate keratinocyte culture. Culturing was performed by a feeder layer method as described previously [17].

Immunofluorescence Immunofluorescence studies were performed on 5-μm cryostat sections, as reported previously [18]. Primary antibodies consisted of GB3 (kindly provided by Professor J-P. Ortonne, Hôpital Pasteur, Nice), LH7:2, and type IV collagen antisera (ICN Immunobiologicals, UK). GB3 antibody labels components of laminin 5 within the lamina lucida [19,20] and has been shown to have attenuated or completely absent staining in junctional forms of EB. LH 7:2 antibody recognizes an epitope within the NC-1 domain of type VII collagen [21]; labeling with this antibody has been shown to be variably reduced or absent in different subtypes of the dystrophic form of EB [22]. Type IV collagen is a ubiquitous component of the lamina densa [23]. These antibodies were diluted 1:2, 1:50, and 1:100, respectively, in phosphate-buffered saline/bovine serum albumin. Labeling was visualized using fluorescein-conjugated rabbit anti-mouse and goat anti-rabbit IgG antibodies (ICN Immunobiologicals, UK) diluted 1:50 in phosphate-buffered saline/bovine serum albumin.

Electron Microscopy Skin samples were processed for electron microscopy using standard methods [24], with primary fixation in half-strength Karnovsky fixative (containing 2% formaldehyde and 2.5% glutaraldehyde in 0.04 M cacodylate buffer), and secondary fixation in 1.3% osmium tetroxide in distilled water.

Immunoelectron Microscopy Skin samples from the affected family members and three control samples from normal skin in unrelated, unaffected individuals were processed for immunoelectron microscopy, using a pre-embedding method. Full details of this technique, including labeling with the GB3 antibody, have been reported elsewhere [25].

PCR Amplification and Heteroduplex Analysis DNA was isolated from peripheral blood lymphocytes by standard methods [26] and used as a template for amplification of genomic sequences within LAMB3. Oligonucleotide primers spanning each of the gene's 23 exons were synthesized on the basis of intronic sequences to generate PCR products [27]. Specifically, to amplify exons 3 and 7 (see results) the following primers were used. For exon 3, the upstream primer was 5′ AATTATTACTGCCAGCAGCG 3′ and the downstream primer was 5′ TACATTTCCTCTTGCCCAAC 3′. For exon 7, the upstream primer was 5′ AGGTTTCTTCTGAACTCGGG 3′ and the downstream primer was 5′ GCAGGGCAAGTATCAAATCC 3′. For PCR amplification, approximately 250 ng of genomic DNA was used as the template in an amplification buffer containing 20 pmol of each primer, 100 nmol $MgCl_2$, 20 μmol of each nucleotide, and 2.5 U of Taq Polymerase (Gibco BRL, Gaithersburg, MD) in a total volume of 50 μl. The amplification conditions for both sets of primers were 94°C for 5 min; then 94°C for 45 seconds; 56°C for 45 seconds; 72°C for 45 seconds, for 40 cycles in an OmniGene thermal cycler (Marsh Scientific Inc). Aliquots of 5 μl of the PCR products were analyzed on 1% agarose gel electrophoresis and 10 μl of the sample was prepared for heteroduplex analysis as per the manufacturer's recommendations (MDE, JT Baker Inc, Phillipsburg, NJ). Staining with ethidium bromide was used to visualize the heteroduplexes. If a heteroduplex was detected, the PCR product was subcloned into the pT7 vector (Novagen, Madison, WI, USA) and sequenced by standard dideoxy nucleotide techniques [28].

Verification of the Mutations The mutations detected in the PCR products (see *Results*) were verified in genomic DNA. The exon 3 mutation resulted in the creation of a new restriction site for the endonuclease *Dde* I. The PCR products spanning this exon were cleaved with this enzyme according to the manufacturers instructions (New England Biolabs). To verify the exon 7 mutation, oligonucleotide probes covering the site of the mutation were designed for ASO of the PCR products encompassing exon 7. A standard ASO protocol was used [29]. The probes were, for the wild type, 5′ AAAAATTCAAGGTCAGTGTGG 3′ and for the mutated allele, 5′ AAAAAATTCAAAGTCAGTGTGG 3′. Seventy-five ethnically matched, unrelated healthy controls were also assessed for the presence or absence of this mutation.

RNA Isolation and Northern Analysis Total RNA was isolated by the guanidium isothiocyanate extraction method [30] from cultured keratinocytes from two affected individuals and one unrelated non-affected control. Approximately 20 μg of total RNA from each sample was electrophoresed on a 1% denaturing gel containing 1.2 M formaldehyde. The RNA was then transferred onto Zeta-Probe blotting membrane (Bio-Rad, Melville, NY). The membrane was hybridized with a ^{32}P-labeled cDNA probe for the β3 chain of LAMB3 [31], as well as with labeled cDNA specific for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [32]. The labeled membranes were then exposed to X-ray film (Hyperfilm MP, Amersham, Arlington Heights, IL) at −70°C and developed after 18–24 h of exposure. The relative mRNA levels were determined using an ultrascan XL laser densitometer (LKB Bromma).

RT-PCR Because the exon 7 mutation (see *Results*) occurred within the splice site consensus sequence at the 3′ end of exon 7 and might therefore have an effect on splicing of the introns, oligonucleotide probes were designed to span the exon borders of the adjacent exons in the affected individuals' cDNA, which was synthesized with reverse transcriptase using approximately 50 μg of total RNA in a volume of 100 μl according to the manufacturer's recommendations (Bethesda Research Laboratories, Gaithersburg, MD). The oligonucleotide probes synthesized were as follows: for exon 6-exon 7 junction, 5′ TGGGGGGAAGGTCCAACTTA 3′; for exon 6-exon 8 junction, 5′ GGGGGGAAGAGGTGGGGG 3′; for exon 7-exon 8 junction, wild type, 5′ AAATTCAAGAGGTGGGGGAG 3′; and for exon 7-exon 8, with mutation, 5′ AAATTCAAAAGGTGGGGGAGA 3′. Dot blotting and probe hybridization were then performed as described elsewhere [29].

RESULTS

Clinical and Genetic Features of the Family All three affected family members, in their 30s when examined, had a history of generalized trauma-induced blistering from birth, which particularly affected the lower legs. In addition to the clinical features shown in **Fig 1**, sparse secondary sexual hair, patchy atrophic alopecia, and albostriate lesions on the trunk were also present. Further clinical details of this family have been described elsewhere [33]. The family contains three affected and three unaffected offspring; both parents have no skin abnormalities. The pattern of inheritance is consistent with an autosomal recessive genodermatosis (see **Figs 5 and 6**).

Laminin 5 Labeling Is Reduced at the Dermal-Epidermal Junction In the affected individuals, GB3 antibody labeling showed faint, yet clearly detectable, linear dermal-epidermal junction fluorescent staining when compared to the bright, continuous linear labeling in the normal control skin **(Fig 2*A,B*)**. Type IV collagen antisera labeling was present along the dermal-epidermal junction and surrounding dermal blood vessels and nerves. In areas of blistering, anti–type IV collagen staining was present along the blister base **(Fig 2*C*)**, consistent with blister formation occurring above the lamina densa. Staining with LH7:2 antibody (anti–type VII collagen) gave bright linear staining at the dermal-epidermal junction in all cases, of similar intensity to normal control skin (not shown). In areas of blister formation this antibody labeled the blister base.

Ultrastructural Preservation of Hemidesmosomes and Anchoring Filaments On transmission electron microscopy, low-magnification examination revealed numerous areas of epidermal-dermal detachment with the plane of cleavage occurring within the lamina lucida **(Fig 3*A*)**. Higher magnification of the hemidesmosome-anchoring filament complex showed no gross structural abnormalities **(Fig 3*B,C*)**. Most hemidesmosomes had well-defined inner and outer plaques. Sub-basal dense plates were often present, and numerous fine anchoring filaments could be seen extending across the lamina lucida from the basal keratinocyte plasma membrane to the lamina densa **(Fig 3*C*)**. Examination of the roof of a blister showed, in some instances, hemidesmosomes with subjacent sub-basal dense plates and wispy anchoring filaments extending into the blister cavity **(Fig 3*D*)**.

Laminin 5 Immunolabeling Is Localized to the Lower Lamina Lucida Subjacent to Hemidesmosomes Ultrastructurally, GB3 immunolabeling in normal human skin was present within the lower lamina lucida just above the lamina densa **(Fig**

Figure 1. Clinical appearances of the affected family members. *A*) A sero-sanguinous blister on the lower abdomen with surrounding erythema and crusting. *B*) Marked involvement of the lower limbs with slow-healing erosions, prominent erythema and some atrophy. *C*) Extensive blistering, scaling, atrophy, erythema, and crusted sero-pustular exudate formation on the lower limb. *D*) Dorsal aspect of the foot showing an inflammatory pustular blister and crusted erosions with almost complete loss of the nail plates from all the toes. *E*) A finger nail showing dystrophic changes with scarring and only a small amount of residual nail plate. *F*) Coalescing pigmented plaques on the inner thigh with scattered blisters and scaling. *G*) Abnormal dentition with extensive enamel hypoplasia, focal pitting, and considerable discoloration of all the teeth. *H*) Marked loss of eyelashes with mild scarring of the lower blepharal margin.

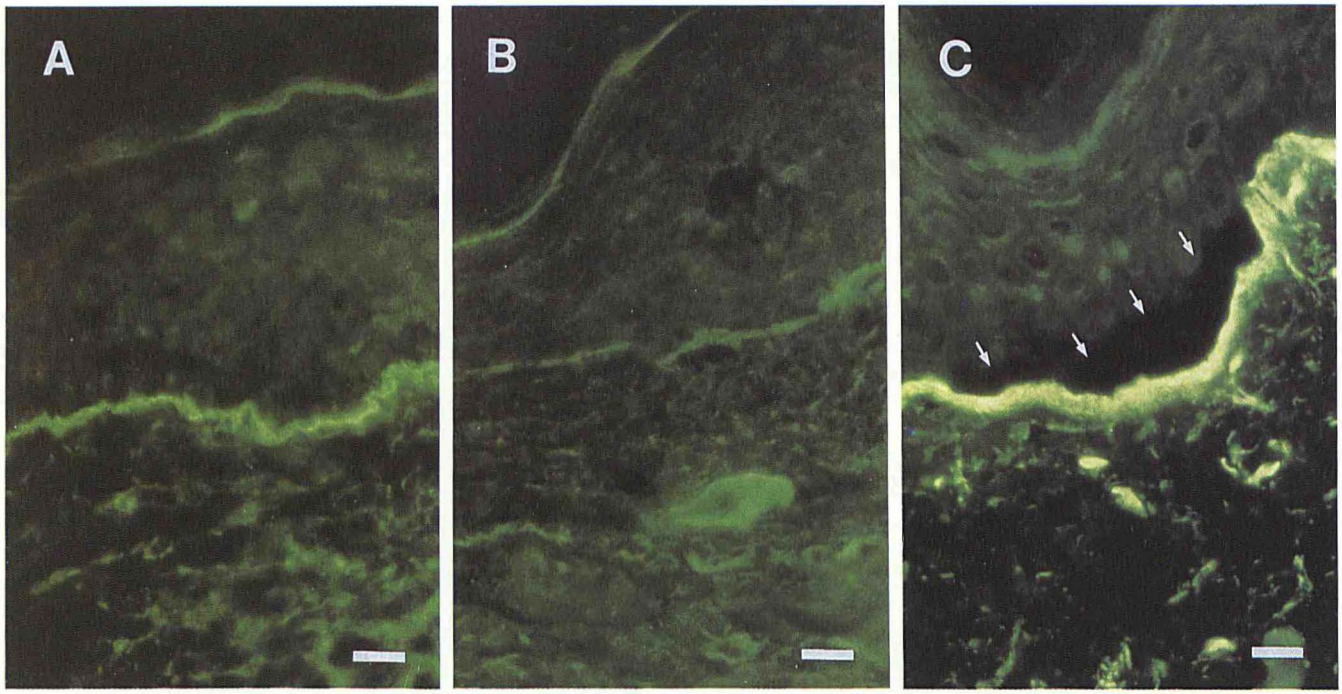

Figure 2. Indirect immunofluorescence microscopy shows that GB3 antibody labeling at the dermal-epidermal junction is reduced. *A*) GB3 antibody staining in normal control skin. There is bright, linear staining at the dermal-epidermal junction. *B*) GB3 antibody staining in patient skin. Reduced intensity fluorescent labeling is present at the dermal-epidermal junction. *C*) Anti–type IV collagen antibody staining in patient skin. Labeling is present at the dermal-epidermal junction, including along the base of a blister, whereas the roof (*arrows*) is unlabeled. (*Bar*, 50 μm.)

4*A*). The labeling was maximal subjacent to hemidesmosomes and was seen in association with the anchoring filaments **(Fig 4*B*)**. In intact patient skin, a reduced amount of similarly distributed immunolabeling was seen **(Fig 4*C*)**, although notably not all anchoring filaments showed GB3 labeling. In areas of separation, some scattered GB3 immunolabeling was detected on the upper border of the lamina densa in the blister base with no associated anchoring filaments **(Fig 4*D*)**. In some areas of patient skin, focal aggregates of immunogold anti-GB3 immunolabeling were present. In such sites, no adjacent normal pattern of immunolabeling was detected **(Fig 4*E*)**.

Identification of the Mutations Within LAMB3 Heteroduplex analysis of the PCR products spanning the 23 exons of LAMB3 in the probands' DNA revealed five distinct shifts. Two of these allelic variants were considered to be pathogenetic in a compound heterozygote **(Figs 5*A* and 6*A*)**. Firstly, nucleotide sequencing of the exon 3 heteroduplex originating from the maternal allele showed the presence of a C-to-T transition at nucleotide position 123 **(Fig 5*B*)**. This point mutation changes an arginine residue (CGA) into a premature termination codon (TGA). Secondly, nucleotide sequencing of the exon 7 heteroduplex from the paternal allele showed a point mutation, with a G-to-A transition at nucleotide position 628 **(Fig 6*B*)**. This change occurs within the last nucleotide of exon 7 and causes a missense mutation, substituting lysine (AAG) for glutamic acid (GAG).

Two additional nucleotide substitutions that did not change the amino acid sequence were detected in exon 6 (384T-to-C, CC<u>T</u> to CC<u>C</u>, proline) and in exon 23 (3432A-to-G, TC<u>A</u> to TC<u>G</u>, serine). These silent polymorphisms have been noted in several other unrelated patients and normal control subjects [34]. Sequencing of the fifth heteroduplex revealed a nucleotide deletion (2702-12delG) within intron 18 but occurring 12 bp away from the consensus splice junction, and therefore unlikely to influence RNA processing.

Verification of the Mutations Within LAMB3 The C-to-T transition in exon 3 was verified by restriction endonuclease digestion using the enzyme *Dde* I (New England Biolabs, Beverly, MA), which creates a new restriction site (C<u>T</u>GAG) in the mutated allele **(Fig 5*C*)**. In the wild type allele, digestion with this enzyme divided the PCR product into fragments 246, 110, and 69 bp in size. In the mutated allele, the 246–bp fragment was further cleaved into 227- and 19-bp fragments. Thus, in the individuals heterozygous for this mutation, i.e., the mother and the three individuals with GABEB, two distinct bands (246 and 227 bp) could readily be appreciated **(Fig 5*C*)**.

The exon 7 mutation did not create or abolish a restriction endonuclease digestion site and was therefore verified by ASO **(Fig 6*C*)**. Only the mother and one unaffected offspring were homozygous for the wild-type allele, whereas the remainder of the family were noted to have both the wild-type and the mutated allele. However, only the three affected individuals had both the exon 3 and exon 7 mutations, thus being compound heterozygotes.

RT-PCR of cDNA derived from cultured keratinocytes from two affected family members did not demonstrate any abnormal splicing of exon 7, despite the point mutation occurring within the splice site consensus sequence. Specifically, ASO across the exon 6–exon 8 border (i.e., for skipping of exon 7) was negative, whereas the normal exon 6–exon 7 borders and exon 7–exon 8 borders were detected. In addition, the missense mutation was noted in the patients' cDNA using the ASO approach.

Northern analysis for the LAMB3 gene **(Fig 7)**. in the affected patients revealed mRNA levels that were reduced to approximately 35% of that in an unrelated, unaffected control subject.

DISCUSSION

There are three main types of epidermolysis bullosa, namely, the simplex, junctional, and dystrophic. Recently, substantial advances have been made in understanding the molecular basis of each category of EB. Specifically, mutations within the genes for keratin 5 and 14 have been reported in EB simplex, and in the type VII collagen gene (COL7A1) in dystrophic EB [35–38]. In junctional EB, the pathogenetic changes appear to be more complex. Three distinct genes encode the laminin 5 polypeptide and mutations have

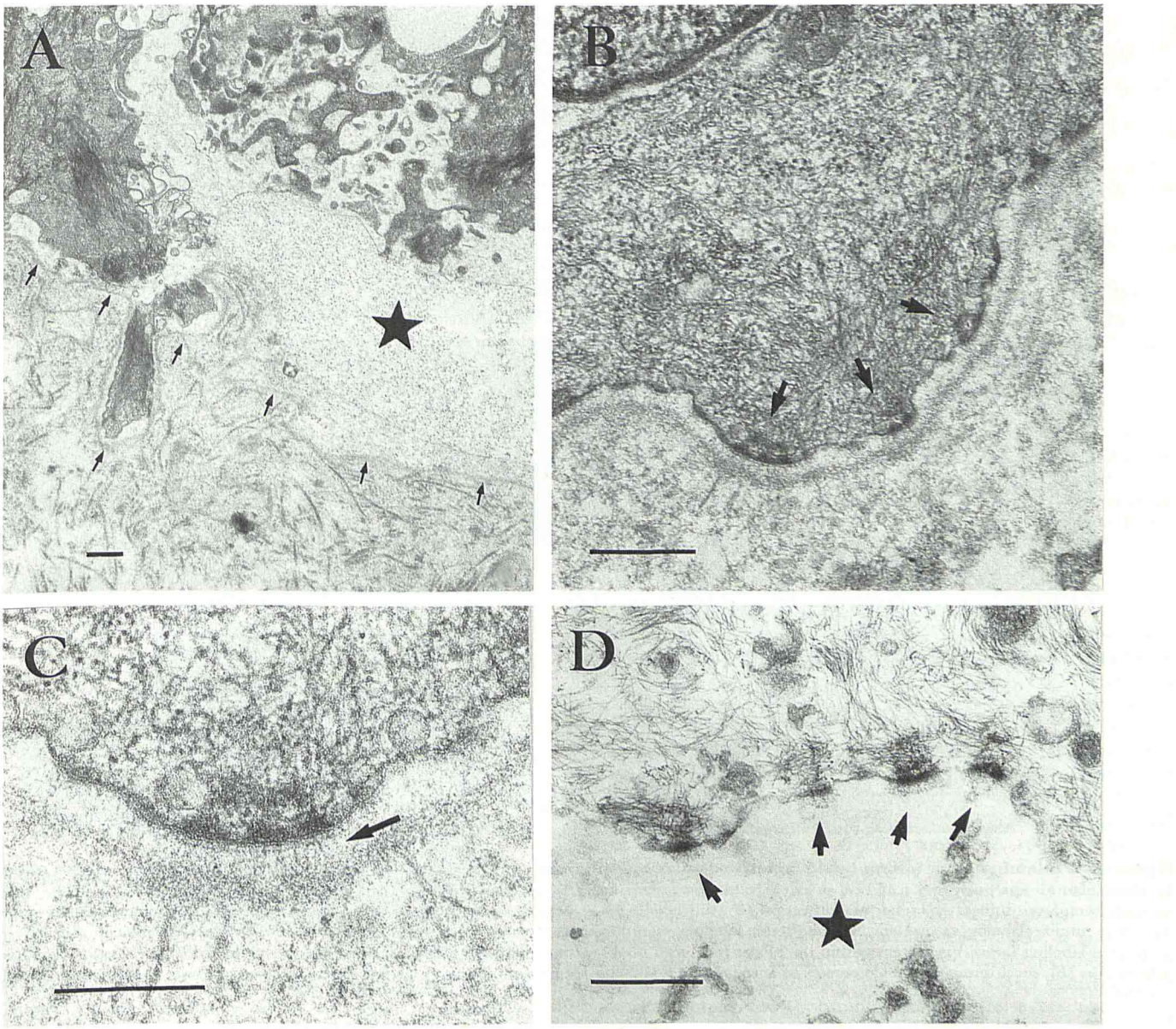

Figure 3. Blister formation within the lamina lucida, and presence of hemidesmosomes and anchoring filaments in intact and separated skin. Transmission electron microscopy at low magnification (*A*) reveals blister formation (*star*) within the lamina lucida. The lamina densa is shown by the *arrows*. At higher magnification (*B*) in intact skin, several well-formed hemidesmosomes (*arrows*) and subjacent anchoring filaments are seen. Inner and outer hemidesmosomal plaques are present (*C*), in addition to sub-basal dense plates and numerous anchoring filaments (*arrows*). In the roof of a blister (*D*), several hemidesmosomes (*arrows*) with brush-like anchoring filaments are seen that extend into the subjacent blister cavity (*star*). (*Bar*, 0.5 μm.)

been described in the genes for the β3 and γ2 chains (LAMB3 and LAMC2, respectively) [14–16]. The cDNA structure for the putative laminin 5 α chain has been published [39], and mutations in the LAMA3 gene have also recently been detected.§¶ The present cases involve LAMB3 mutations, including the first missense mutation within the β3 chain. Apart from laminin 5, there is some evidence to suggest that other candidate genes such as that encoding the 180-kD bullous pemphigoid antigen (BPAG2) may also be involved in the pathogenesis of certain forms of junctional EB [40]. Likewise, other candidate genes including those for the basal cell integrins, α6 and β4, might also be implicated [41].

In the original GABEB family reported from Austria [4], electron microscopy showed a reduced number of hemidesmosomes and associated anchoring filaments. However, variability in hemidesmosome morphology has been documented in other GABEB patients [7]. It is interesting to note, therefore, that the hemidesmosome-anchoring filament complexes in our patients were well maintained ultrastructurally, despite the considerable overlap in the clinical appearances of all the reported GABEB patients. Of further interest is the observation that, in the Austrian family, GB3 antibody labeling of the dermal-epidermal junction showed normal intensity staining** in contrast to the patients in our study. Furthermore, labeling with a 180-kD bullous pemphigoid antigen

§ Baudoin C, Aberdam D, Christiano AM, Uitto J, Ortonne J-P, Meneguzzi G: Impaired expression of the LAMA3 gene causes Herlitz junctional epidermolysis bullosa (abstr). *J Invest Dermatol* (in press).

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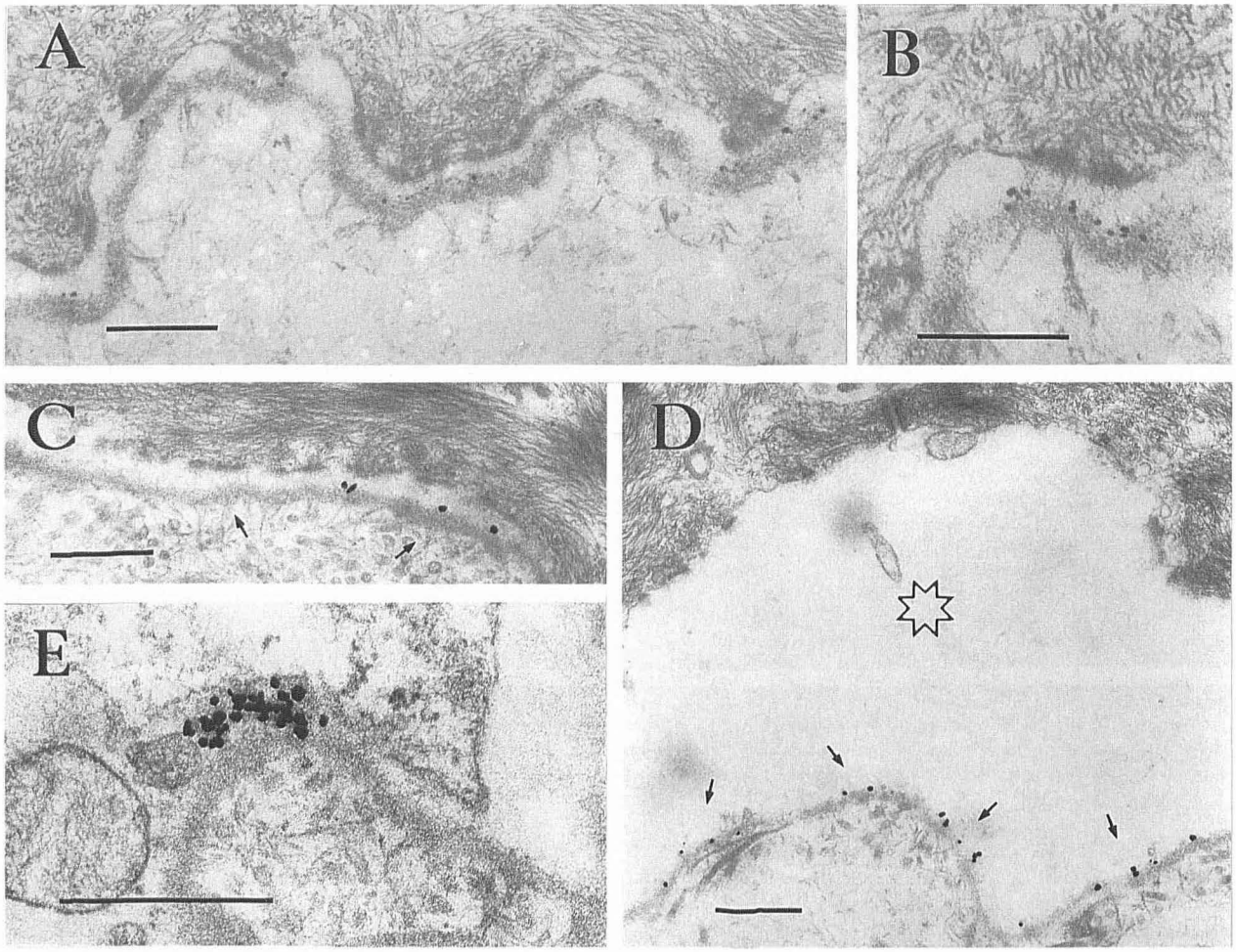

Figure 4. Pre-embedding immunogold electron microscopy (1-nm gold with silver enhancement) using the GB3 (anti–laminin 5) monoclonal antibody on unfixed skin. In normal control skin (*A*), extensive labeling is present within the lower lamina lucida, particularly beneath hemidesmosomes. At higher magnification (*B*), this labeling is associated with anchoring filaments just above the lamina densa. In intact patient skin (*C*), similar labeling is present, although the labeling is reduced and patchy (compare arrowed sites). In blistered patient skin (*D*), the immunogold labeling (*arrows*) is present within the blister base with no associated anchoring filaments. The blister is depicted by the *asterisk*. In some areas in patient skin (*E*), focal immunogold aggregates of anti–laminin 5 labeling are present with no adjacent normal pattern of lower lamina lucida labeling. (*Bar*, 0.5 μm.)

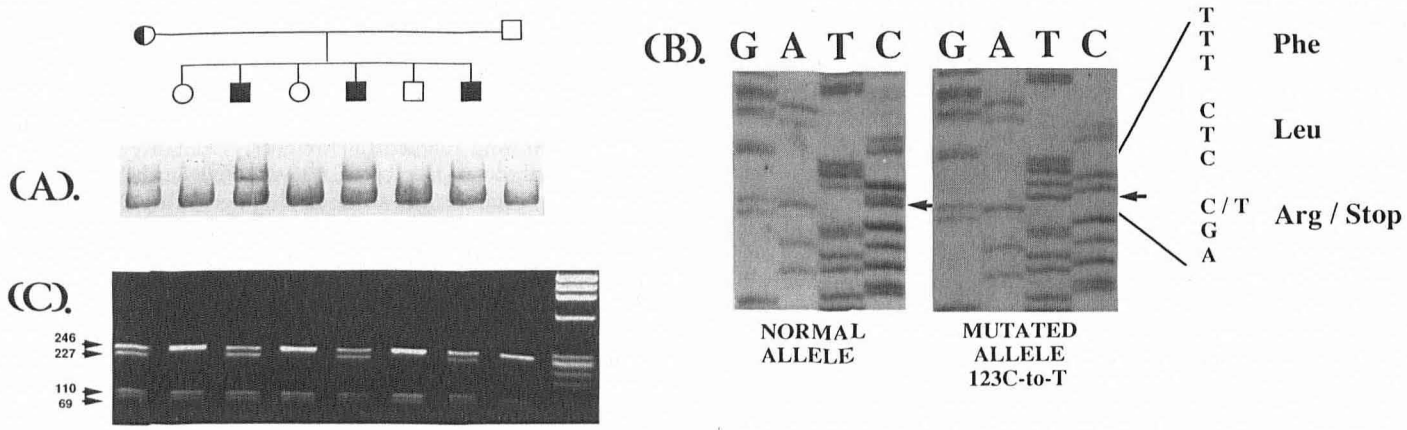

Figure 5. A nonsense mutation within LAMB3 exon 3. Heteroduplex analysis (*A*) shows band shifts in the three affected individuals and in the maternal DNA. Nucleotide sequencing (*B*) reveals a C-to-T substitution (*arrows*) that changes an arginine residue (CGA) into a premature termination codon (TGA). Verification of the mutation (*C*) with *Dde* I restriction endonuclease digestion of the PCR products spanning exon 3 results in two distinct bands (246 and 227 bp) in the mother's and three affected individuals' samples, and a single 246-bp band in the other unaffected family members. Smaller digestion products of 110 and 69 bp are detected in each individual.

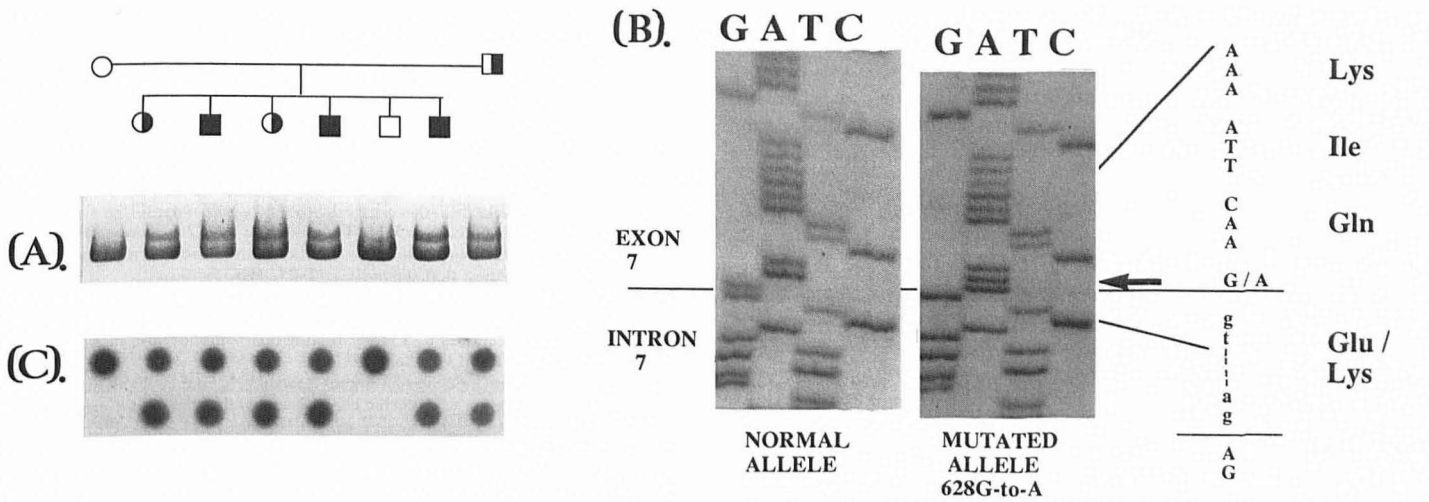

Figure 6. A missense mutation within LAMB3 exon 7. Heteroduplex analysis (*A*) shows bandshifts in the three affected individuals DNA and also in the DNA from the father and two unaffected siblings. Nucleotide sequencing (*B*) reveals a G-to-A substitution (*arrows*) in the last nucleotide of exon 7. This is the first nucleotide of a split codon for a glutamic acid residue (GAG) that is substituted by lysine (AAG) in the mutated allele (*arrows*). Verification of the mutation (*C*) by ASO with a wild-type probe (*top row*) and a mutated allele probe (*bottom row*) that span the mutation. Only the mother and one unaffected sibling are homozygous for the wild-type allele. The remaining family members, including the three siblings with GABEB, are heterozygous for the nucleotide substitution.

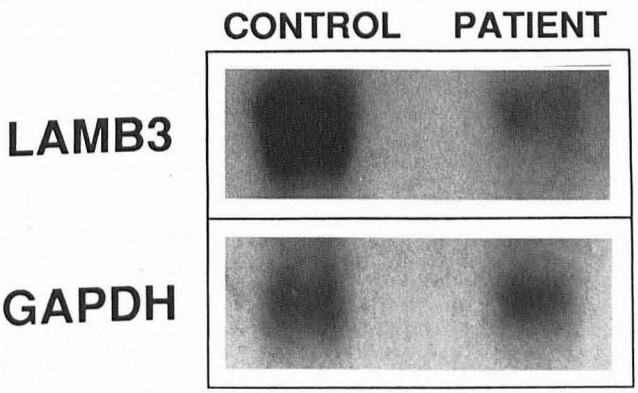

Figure 7. Reduced mRNA levels in an affected individual. Northern blotting shows that the LAMB3 signal in the patient is reduced to approximately 35% of that of the control signal intensity. Similar GAPDH labeling is present in both lanes signifying equivalent RNA loading.

antibody showed reduced staining in the Austrian patients,†† whereas labeling was of normal intensity in our patients (data not shown). These observations suggest that abnormalities in several different adhesion molecules at the dermal-epidermal junction may give rise to JEB phenotypes, including those with clinical features of GABEB.

Analysis of the mutations so far reported in EB reveals that many of the severe forms of the disorder, both junctional and dystrophic, involve the creation of a premature termination codon (PTC). Such mutations may lead to truncated polypeptides, and the PTC found near the 5′ end of the mutated maternal allele (exon 3) in the present case would be expected to result in a severely truncated, non-functional protein. However, apart from merely truncating the peptide, it would appear that the main effect of this type of mutation is to create an unstable mRNA transcript [42,43]. The Northern analysis presented here is consistent with such a hypothesis.

†† Pohla-Gubo G, Lazarova Z, Giudice G, Liebert M, Grassegger A, Hintner H, Yancey K: Diminished expression of the extracellular domain of bullous pemphigoid antigen 2 (BPAG2) in the epidermal basement membrane (BM) of patients with generalized atrophic benign junctional epidermolysis bullosa (GABJEB) (abstr). *Clin Res* 42:454A, 1994.

The missense mutation within exon 7 appears to be of pathogenetic importance for several reasons. Firstly, it causes a significant polarity change from a negatively charged glutamic acid to a positively charged lysine residue. Secondly, the mutation occurs within part of laminin 5 that may be critical for structural interactions with other basement membrane zone macromolecules. Thirdly, the mutation could not be demonstrated in 150 unrelated chromosomes within an ethnically matched control population. This mutation occurred within the last base pair of exon 7 and might therefore also have an effect on RNA splicing. Exon 7 contains 64 base pairs and consequently skipping of this exon would be out of frame leading to a PTC 22 base pairs downstream from the mutation (TGA, at nucleotide position 650-652). Taken in conjunction with the maternal mutated PTC allele, if such exon skipping would have occurred, the affected family members might be expected to have little, if any, stable LAMB3 transcript, no functional protein, and almost inevitably a more severe, potentially lethal, phenotype. However, there was no evidence of any abnormal splicing of exon 7 on RT-PCR assessment and the missense mutation was detected within the cDNA by ASO.

The exon 7 missense mutation occurs within domain VI of the molecule. In classical laminin (laminin 1), the globular amino-terminal domain VI within the $\beta 1$ chain appears to have an important role in the self-assembly of laminin molecules *in vitro* [44,45]. In laminin 5, the corresponding domain VI, after post-translational processing, is present only within the $\beta 3$ chain, whereas the $\alpha 3$ and $\gamma 2$ chains are truncated in comparison with the $\alpha 1$ and $\gamma 1$ chains in laminin 1. Thus, domain VI of the $\beta 3$ chain may be important in the association of laminin 5 with other laminin molecules within the extracellular matrix. Assessment of the amino acid sequence of this part of the $\beta 3$ chain gene (LAMB3) reveals some functional differences with other laminin β chains, including an odd number of cysteine residues [31]. It is possible that this part of the molecule interacts with the short arm of the laminin 6 (K-laminin) α chain [46] and, in this way, contributes to the structural integrity of the dermal-epidermal junction. Such an association may be disrupted by the missense mutation within the mutated paternal allele in this particular family. The immunogold electron microscopy findings of focal anti-GB3 aggregates within the lamina lucida could also be consistent with laminin 5 $\beta 3$ chains containing the mutation being secreted into the extracellular space but then being unable to associate with other basement membrane zone macromolecules.

In summary, this study has demonstrated the presence of both nonsense and missense mutations within the LAMB3 gene in three siblings with a non-lethal form of junctional epidermolysis bullosa. The results are of value in elucidating the pathogenetic mechanisms of this inherited skin disorder, and also in attempting to understand the complexities of adhesion between the epidermis and the underlying dermis.

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